THE DNA-DEPENDENT INCORPORATION OF RIBONUCLEOTIDES INTO RNA IN THE CHICKEN EMBRYO*

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The deoxynucleic acid directed synthesis of ribonucleic acid, catalyzed by the enzyme RNA polymerase, has been demonstrated to occur in bacteria, a variety of animal tissues, and pea seedlings (Hurwitz and August, 1963; Weiss, 1960; Smellie, 1963). The RNA polymerase obtained from animal sources has usually been obtained as an "aggregate", sedimentable at low centrifugal force and bound to DNA active as primer. DNA appeared to be necessary as the reaction was DNAse sensitive (Baltimore and Franklin, 1962) and, with an enzyme obtained from ascites tumor cells, the addition of DNA was required for maximum activity (Smellie, 1963; Cline, Eason, and Smellie, 1963). The present communication is a report on the RNA polymerase of chicken embryos. Obtained from this source, and partially purified, the enzyme is not only dependent on DNA for maximum activity but, in addition, the deoxynucleotide composition of the DNA added to the reaction influences the rates at which the ribonu-

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cleotides are incorporated. The enzyme is soluble and, to the extent examined, the characteristics of the reaction parallel the characteristics of the reaction catalyzed by the bacterial enzyme.

The extract used was obtained by mechanically disrupting 12-13-day-old embryos (sans heads) in a Waring Blender in a solution containing 0.05 M Tris buffer pH 7.5; 0.005 M Mg Cl_2; 0.005 M 2-mercaptoethanol and centrifuging the extract at 78,000 x g for 90 minutes. The enzyme activity was purified from the 78,000 x g supernatant solution approximately 10-fold by precipitation with protamine sulfate, elution with 0.25 M succinate buffer pH 6, followed by ammonium sulfate fractionation (0 - 40%).

This fraction has the characteristics summarized in Table I. Incorporation of UMP is stimulated approximately 7-fold by the addition of DNA. Omission of one of the nucleoside triphosphates or the addition of DNAse or RNAse markedly reduces incorporation. Actinomycin D, a relatively specific inhibitor of RNA polymerase (Hurwitz et al., 1962a), also markedly reduces UMP incorporation.

To determine the nature of the product of the reaction, a nearest-neighbor experiment was performed with αP^{32}-UTP as substrate. The components of a reaction mixture such as described in the legend to Table I were increased in order to obtain a product containing 1 mμmole of UMP^{32}. This material was subjected to alkaline degradation and the mononucleotides separated by electrophoresis (Furth et al., 1961). The distribution of radioactivity (AMP, 23%; UMP, 33%; GMP, 22%; CMP, 22%) indicates that UMP is incorporated adjacent to all four ribonucleotides. In a parallel experiment, with C^{14}-UTP as substrate, the material was subjected to alkaline

TABLE I

Requirements for UMP incorporation with Thymus DNA as primer

Additions	μμmoles incorporated
1. Complete	75
2. Omit DNA	11
3. Omit ATP	16
4. Omit CTP	19
5. Omit GTP	16
6. Omit ATP, CTP, and GTP	19
7. Complete + Actinomycin D (5 μg)	20
8. Complete + DNAse (25 μg)	4
9. Complete + RNAse (25 μg)	9

The complete reaction mixture (0.5 ml) contained: 80 μM αP^{32}-UTP (9.5 x 10^6 cpm/μmole); 160 μM each of CTP, GTP, and ATP; 8 mM $MgCl_2$; 2 mM 2-mercapto-ethanol; 50 mM Tris buffer, pH 7.7; calf thymus DNA equivalent to 64 mμmoles of deoxynucleotide, and 300 μg of the ammonium sulfate fraction. After incubation for 20 minutes at 38°, the reaction was terminated by the addition of 0.2 ml of 7% perchloric acid, albumin (0.5 mg) added, and the acidified mixture centrifuged. The precipitate was washed twice with 3 ml portions of 1% perchloric acid dissolved in 1.5 ml of 0.2 N NH_4OH, decanted into metal planchets, dried, and the radioactivity measured in a windowless Geiger-Müller counter.

degradation and UMP and uridine isolated by paper chromatography in isobutyric acid/concentrated NH_4OH/water (66/1/33). 70% of the radioactivity was recovered as UMP and 21% as uridine, indicating that UMP was predominantly incorporated internally in the synthesized RNA.

The DNA used in the reaction influences the rates at which each nucleotide is incorporated. As shown in Table II, when DNA

TABLE II

Influence of DNA from Different Sources on the Incorporation of Ribonucleotides

DNA Source	Nucleotide Incorporation in μμmoles				RNA		DNA	
	AMP	UMP	CMP	GMP	$\frac{A+G}{C+U}$	$\frac{A+U}{C+G}$	$\frac{A+T}{G+C}$	
Calf Thymus	145	155	125	107	0.90	1.29	1.25	*
Chicken Embryo	54	64	33	33	0.90	1.79	1.23	**
M. lysodeikticus	94	78	225	233	1.08	0.38	0.39	***
dAT copolymer	28	36	< 1	< 1	0.78	>40.	>40.	*

The reaction mixtures and assay were as described in the Legend to Table I, except as follows: 1) 160 μM labeled substrate was used. The specific activities (cpm/μmole) of the labeled nucleotides were: C^{14}-ATP, 5.7×10^6; C^{14}-UTP, 2.0×10^6; C^{14}-CTP, 6.1×10^6; C^{14}-GTP, 5.8×10^6. In the reactions primed with dAT copolymer, P^{32}-UTP (specific activity 9.5×10^6 cpm/μmole) was used. 2) The amounts of DNA added (expressed as mμmoles of deoxynucleotide) were: calf thymus, 64; chicken embryo, 170; M. lysodeikticus, 38; dAT copolymer, 3.8. 3) 700 μg of the ammonium sulfate fraction was added except in the dAT copolymer primed reactions where 300 μg were added.

The results reported are the differences between the incorporation of labeled precursor in the presence and absence of DNA. In the absence of DNA, the incorporation (μμmoles) of labeled substrates were: C^{14}-AMP, 8; C^{14}-UMP, 13; P^{32}-UMP, 18; C^{14}-CMP, 13; C^{14}-GMP, < 1.

* Lehman et al., 1958.
** Smith and Stoker, 1951.
*** Lee et al., 1962.

isolated from calf thymus or chick embryo is present, the $\frac{A+T}{C+G}$ ratio is considerably higher than when DNA of M. lysodeikticus is present. When dAT copolymer is used as primer, incorporation of AMP and UMP is significantly increased, while the incorporation of CMP and GMP is unaffected.

These results indicate that the RNA polymerase present in chicken embryos can be isolated in soluble form and can be partially freed of DNA active as primer. As is the case with the bacterial enzyme (Hurwitz and August, 1963; Hurwitz et al., 1962b), the deoxynucleotide composition of the DNA added to the reaction influences the rates at which the ribonucleotides are incorporated.

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